thought to provide the motive force for epiboly, are defective in MZ*eomesa* mutant embryos. Prior to doming, the yolk microtubules are abnormally bundled, leaving large regions entirely devoid of microtubules. Importantly, both the doming delay and the yolk microtubule distribution are rescued by injection of *eomesa* mRNA into embryos at the 1-cell stage. In addition to the yolk defects, the deep cells of the blastoderm display abnormal morphologies. The deep cells are more tightly packed and exhibit more bleb-like protrusions than cells in control embryos. Transplantation studies are being conducted to determine if the deep cell defects are cell autonomous or non-cell autonomous. Our continued investigation of the basis of the defects in MZ*eomesa* mutant embryos should provide new insights into the molecular control of epiboly. Eomesodermin has also been implicated in gastrulation movements in both *Xenopus* and mice, pointing to a conserved role in regulating morphogenesis.

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Program/Abstract # 114
The cytoplasmic tyrosine kinase Arg regulates Xenopus gastrulation via the adaptor protein CrkII
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Coordinated cell movements during vertebrate gastrulation are crucial for correct placement of embryonic tissues along body axes and are controlled by multiple signals. While non-canonical Wnt pathway is shown to regulate cell polarity and directional cell behaviors via the cytoplasmic protein Dishevelled, the mechanisms used by receptor tyrosine kinases, such as PDGFR, FGFR and ErbBs, to modulate gastrulation are less understood. Here, we show that the actin-binding cytoplasmic tyrosine kinase Arg modulates cell movements during Xenopus gastrulation. Arg was expressed in dorsal tissues at the onset of gastrulation, and both gain- and loss-of-function of Arg disrupted gastrulation movements and led to defective frog tadpoles. Overexpression of Arg inhibited head mesoderm migration effectively, while reduction of Arg by specific antisense morpholino oligos caused aberrant head mesoderm migration, resulting in reduced migratory distance and increased cell dissociation. Both overexpression and depletion of Arg also affected convergent extension movements. The regulation of Xenopus gastrulation by Arg required an intact kinase domain, but the actin-binding motif could be dispensed. Arg controlled phosphorylation of endogenous CrkII, an adaptor protein involved in activation of Rho family GTPases and actin reorganization. Our data thus imply that Arg may be an essential mediator of receptor tyrosine kinases during gastrulation and can modulate cell movements via phosphorylation of an important effector CrkII.

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Program/Abstract # 115
Fritz regulates the membrane stability mediated by septins dynamics during Convergent Extension in Xenopus embryo
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The Planar Cell Polarity (PCP) pathway is acritical regulator for cell behaviors during development. Although there isaccumulating data showing that core PCP proteins are necessary for cellpolarity, much less is known about how individual cells respond to PCP signaland change their behavior. Fritz is one of the PCP effector proteins, whichacts downstream of the core PCP proteins to control specific processes in *Drosophila*. We investigated the function of Fritz to unveil the processbetween core PCP and the changing of cell behavior during Convergent Extensionin *Xenopus* embryos. We found that Fritz wasexpressed in the dorsal mesoderm, and GFP fused Fritz localized at the cellmembrane. Inhibition of Fritz function using antisensemorpolino-oligonucleotides (MO) lead to the gastrulation defects and abnormalcell membrane dynamics (undulation and appearance of blebs). We found thatFritz physically interacted with septins, cytoskeletal elements that providecortical rigidity. Septins–MOs caused blastopore closure and cell behaviordefects similar to Fritz-MO. Also, GFP-fused septins localized in or near thecell membrane depending on Fritz. Importantly, the cell elongation wasattenuated in all these morphants, but the medio-lateral polarity wasmaintained as in wild type embryos. From these results, we conclude that Fritzregulates septins, as the executants of the PCP pathway to control the membranestability and cell elongation during Convergent Extension.

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Program/Abstract # 116
Serotonin and Wnt signaling are required for morphogenesis of the gastrocoel roof plate epithelium, the site of symmetry breakage in the frog embryo
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Organ laterality in vertebrates results from asymmetric signaling in the embryo. Symmetry breakage in fish, amphibian and mammalian embryos depends on cilia-driven flow of extracellular fluid during neurulation. In *Xenopus* a functionally relevant asymmetry of serotonin localization was postulated already at the 16-cell stage. We report the role of serotonin signaling in the context of flow. Flow, and consequently asymmetry, were lost in embryos in which serotonin signaling was downregulated, either in receptor morphants or by sequestration of extracellular serotonin upon expression of a secreted serotonin-binding domain. Serotonin signaling was required for the specification of the ciliated gastrocoel roof plate (GRP) epithelium during gastrulation, the site of leftward flow. A second pathway involved in this process is canonical Wnt signaling, as shown by flow and laterality defects in receptor (fz8) morphants. Our data suggest that serotonin acts as a permissive and Wnt as an instructive signal to specify the GRP.

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Program/Abstract # 117
Development of swimming regulation systems in sea urchin: From blastulae to larvae
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In sea urchin embryos, motile cilia are evident from the blastula stage, and rotatory movement by embryos can be observed in the fertilization envelope. Serotonin plays a role in the regulation of the beating of larval cilia. The serotonergic nervous system is yet to appear in blastulae. Thus, the regulation system of cilia of blastulae is unknown. Nevertheless, the swimming behavior of blastulae is organized to a considerable degree. The beating of cilia is regulated also by dopamine (DA) in invertebrates and

vertebrates. In sea urchin, DA and its receptor DRD1 were detected from the period when the embryos acquire rotatory movement in the unhatched blastula stage, indicating dopaminergic system is a likely candidate that is involved in the ciliary beating in blastulae. Immunohistochemically DA and DRD1 were detected associated with a few micrometer diameter granules, and they were closely localized with γ-tubulin at the base of cilia. Inhibition of DA synthesis or knockdown of *DRD1* gene resulted in severe decreasing of swimming activity in blastulae. During larval development, the ciliary band emerged evidently associated with local cell proliferation and the change of ciliary ectodermal cell shape on the larval arms and the anterior and posterior epaulets. The serotonin receptor cell network and serotonergic nervous fibers closely fringed the ciliary bands of the anterior and posterior epaulets and of the larval arms. The present observations provided the structural basis of our previous observation that larval swimming activity is less sensitive to serotonin deprivation in older larvae than younger blastulae.

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Program/Abstract # 118
Serotonin signaling initiates gastrulation in the sea urchin
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Gastrulation in the sea urchin begins with vegetal plate invagination. Pharmacological studies from our lab suggest that 5-HT triggers the gastrulation process. However, the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH), has been reported to not be expressed until the late gastrula stage, and only in cells of the developing serotonergic nervous system; furthermore an inhibitor of TPH, p-chlorophenylalanine (p-CPA), did not block gastrulation. This calls into question the role of serotonin in the gastrulation process. In the present study we show that methyl-p-CPA blocks gastrulation *in vivo* and tryptophan hydroxylation *in vitro*. Serotonin was identified in vegetal plate and primary mesenchyme cells in mesenchyme blastula and early gastrula embryos prior to localization in serotonin neuron precursors. We demonstrate that preneural embryos utilize a different enzyme, phenylalanine hydroxylase/tryptophan hydroxylase (PAH/TPH), than that (TPH) used in developing larval serotonergic neurons to hydroxylate tryptophan, and PAH/TPH mRNA is present in blastula and gastrula stage embryos. Vegetal plate invagination blocked by methyl-p-CPA can be rescued by co-incubation with serotonin, as well as with agonists and downstream effectors of type 2 (DOI, PMA) and type 7 (8-OH, cyclic AMP) serotonin receptors. The direct activator of protein kinase A (PKA), cyclic AMP, demonstrated the greatest rescue effect, and methyl p-CPA inhibits phosphorylation of PKA and, to a lesser extent, protein kinase C. This study demonstrates that serotonin acts on type 2 and/or type 7 receptors to initiate gastrulation in the sea urchin.

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Program/Abstract # 119
Dissecting Tentacle Formation in Hydra Using Chemical Genetics
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In the basal metazoan *Hydra*, tissues are in a state of constant growth and replacement. Thus, developmental processes such as morphogenesis and differentiation are continuously active in the adult *Hydra* and are orchestrated by ongoing signal transduction. Classical genetic approaches to dissect developmental pathways are difficult to carry out with *Hydra*. We have initiated a small molecule screen to better understand regeneration and maintenance of *Hydra*'s simple body plan. We have identified a small molecule, DAC-2-25, that expands the existing tentacle zone in a progressive and polar fashion. Using phylogenetic profiling we have identified strains that respond to DAC-2-25 (e.g. *Hydra vulgaris* strain AEP), or that don't respond, (e.g. *Hydra vulgaris* strain Zurich). We have used chimeras of these strains to identify the responding tissue layer. Transgenic *Hydra* expressing fluorescent proteins under the control of relevant promoters are being used to examine how DAC-2-25 perturbs tentacle formation. Structure-activity relationship studies have identified the features of DAC-2-25 that are required for activity. Ultimately, we plan to identify the protein target of DAC-2-25 by using a combination of affinity chromatography and bulk segregant analysis of progeny from a cross between responding and non-responding strains.

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Program/Abstract # 120
Nodal signaling is involved in left-right asymmetric ocellus formation in Ciona intestinalis
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Nodal signaling plays an essential role in establishment of left-right asymmetry in a variety of animals. In the ascidian, *Ciona intestinalis*, left-right asymmetry can be observed e.g. in the coiling of the elongating tail of tailbud embryos in the chorion, positioning of the sensory pigment cells in the larva and gut formation in the juvenile, and *Nodal* is expressed on the left side of the sensory vesicle (SV) and epidermis in the developing tailbud embryo. However, it is largely unknown how Nodal signaling is involved in establishment of left-right asymmetric morphology. To address the involvement of the left-sided Nodal signaling in the establishment of asymmetric morphology in the development of *C. intestinalis*, we analyzed effect of the inhibition of Nodal signaling on the formation of the ocellus pigment cell, located on the right side of the SV of the larva in normal development. Upon the inhibition of Nodal signaling with the inhibitor, SB431542, the ocellus pigment cell was located on the midline, and melanin granules in the cell were separated by the midline. Moreover, *Ci-opsin1*, a marker gene of the ocellus photoreceptor cells expressed on the right side of the SV in normal development, was ectopically expressed on the left side as well as on the right side of the SV. Likewise, *Ci-Rx* that is required for ocellus differentiation and is expressed on the right side of the SV in normal development was expressed bilaterally upon the inhibition of Nodal signaling. These results suggest that the left-sided Nodal signaling controls the asymmetric ocellus formation in the larval development of *C. intestinalis*.

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Program/Abstract # 121
Post-intercalation elongation and narrowing of the ascidian notochord requires actomyosin contractility and endocytosis
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The control of cell shape is of central importance in morphogenesis, but issues of scale and resolution make it challenging to characterize these shapes across entire tissues and organs. We have developed a computer-assisted method to reconstruct the three-dimensional shape of every cell in the developing notochord of the simple chordate *Ciona savignyi* based on confocal microscopy of phalloidin-stained embryos.